Storage stability and color characteristics of acylated and non-acylated anthocyanins in fish and beef gelatin gel system

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Abstract

Gummies are well-known confectionaries usually dyed with artificial colorants. However, some health concerns about artificial colorants have pushed the industry to produce gummies with naturally derived colors. Anthocyanins are pigments in vegetables and fruits, giving colors from red to purple and blue depending on pH. The source of gelatin is a concern in Jewish and Muslim communities since gelatin usually made by pork. Our objective was to determine the color characteristics and pigment stability of anthocyanins in gummies made by beef and fish gelatin.

Gummies were prepared from beef (BG) and fish gelatin (FG) using published formulations with slight modifications, and colored by chokeberry and American elderberry (AE) extract, with anthocyanin concentration of 43.24 mg/L and 32.14 mg/L in the gelatin respectively. The color performance and the anthocyanin concentration in the fish and beef gelatin were monitored weekly with a Hunter ColorQuest and a Shimadzu spectrophotometer, respectively. Anthocyanins in gelatin were extracted using ethanol.

No significant changes on the lightness were observed in chokeberry or AE dyed fish or beef gel. However, there were a significant decrease in the anthocyanin concentration in both anthocyanin sources. The anthocyanin concentration of FG colored by chokeberry extract degraded 57% after a 24-days storage in dark, RT ($P = 0$), and degraded 56% in BG under the same storage condition ($P = 0$). While samples colored by American elderberry, which high in acylated anthocyanin, only degraded 15% and 19% in FG and BG, respectively, after a 36-days storage under the same condition. Overall, the anthocyanins from AE seems more stable than chokeberry anthocyanin in both BG and FG, while the BG was more stable in FG for American elderberry ($P=0$).

This experiment can help to determine the color performance of anthocyanins in FG and BG for commercial production of gummies with no synthetic colors, and to find out an alternative for pork gelatin as well as compare the different anthocyanin sources' effectiveness.

Introduction

In recent decades, scientists have investigated the potential of anthocyanins as natural colorants, meanwhile, the customers are also aware of the benefits of natural ingredients including natural colorants (Li and others, 2017). The food industry has realized the importance

to attract consumers with “natural” products and gotten benefits from it. There is a forecast that shows an increase in natural food colors due to the high demand for confectionary and bakery goods, and the global market size for natural colors grows rapidly since 2015 (Grand View Research, 2017). The potential health benefits of anthocyanins have been proved by plenty of researches. For instance, Li and others (2017) pointed out the anticancer activity, anti-inflammatory activity, anti-obesity, and many others of the potential benefits of anthocyanin.

Based on the presence of acids on sugars, anthocyanins can be classified as non-acylated and acylated. The acylated anthocyanins are generally more stable than the non-acylated ones due to the chemical structure arrangement (Willig, 2009). The American elderberry (*S. canadensis*) which exist in temperate and subtropical region has about 80% acylated anthocyanin which is rare high among fruits (Lee and Finn 2007). Chokeberry (*Aronia melanocarpa*), has the non-acylated anthocyanin which mainly comprised of cyanidin-3-glucoside contains proanthocyanidins which possess anti-radical effect (Christian and others, 2015).

Gummies is popular among children and teenagers because of the eating pleasure. Gelatin is a gelling agent used often in gummies. Gelatin is a digestible protein produced from the partial hydrolysis of collagen and is the combination of the proteins and peptides mixed (Jeya and others, 2012). This water soluble polymers has a lot of functions including thickening, gelling as well as stabilizing agent (Imeson, 1997). Gelatin can come from the skin, bones, or white connective tissues of pork, beef and fish. Usually those people with religious restrictions choose the beef or fish as the source of gelatin. The difference on color between the fish and beef gelatin is most prominently in the light intensity when studied in film form. Fish gelatin has higher lightness values compared to beef gelatin (Nur and others, 2012). That results from the yellowish hue of beef gelatin powder compared to the whitish color of fish gelatin powder.

Although natural colorants, especially anthocyanins, have been applied in commercial gummies by certain manufacturers such as Simply Balanced, Lunch Buddies and Black Forest Organic Gummy Bears, limited studies have been done on the anthocyanins’ color performance in different sources of gelatin. Thus, the focus of this experiment was to study the influence of beef and fish gelatin on different acylation type of anthocyanins in term of color characteristics and stability. This study is important for industry to choose an alternative of pork gelatin.

Hypothesis

Our hypothesis is that different sources of gelatins can influence the anthocyanin color characteristics and stability. Also, we hypothesis that acylated anthocyanins can perform a higher stability and different color characteristics compared with the non-acylated ones, because of their intermolecular and intramolecular interaction.

Methods

After harvesting, chokeberry and American elderberry samples were stored at -18°C until extraction. About 50g of each was homogenized with liquid nitrogen in the blender. The powder was then vacuum filtered and re-extracted with aqueous acetone until a clear solution was obtained. Then extraction solution was mixed with same amount of chloroform in a separatory funnel, and stabilized for at least 2 hours. The anthocyanin portion was collected and evaporated to remove residual organic solvents. The final extracts was taken to a known volume using acidified water. Anthocyanin concentration was determined by pH differential method (Giusti and Wrolstad, 2001).

The preparation of the model gelatin was follow Maier and others' recipe (2009). 20g of fish or beef gelatins was mixed with 78g water and heated to 75° C for 2 minutes. 97.25g sucrose was dissolved in 38g water, heated to 115°C and combined with previous gelatin solution. Additionally, 0.3g of sodium benzoate was added as preservative. Finally, 14.85 ml and 11.48ml of chokeberry and AE was added respectively to the 250ml of gelatin solution after cooling down. Then, the colored gelatins were put in molds and stored in dark under room temperature (around 20 ° C) for further analysis.

Color characteristics (L*, a*, b*, C*, H*) of each samples was measured by Hunter ColorQuest colorimeter in certain time period over 24 days for chokeberry and 36 days for AE, each done with three replications. Anthocyanins in samples were also re-extracted by dissolving in acidified hot water before precipitating the gelatin at pH 5 (isoelectric point) with 240mL of ethanol. Then it was centrifuged for 10 minutes at 4000rpm to remove the solids while the supernatant was evaporated for anthocyanin recovery for quantification by the Shimadzu spectrophotomer. The quantification data also were collected over 24 days for chokeberry and 36 days for AE.

Results and Discussion

Two hours after mixing with chokeberry extract, the color of the fish and beef gummies were in the red to yellow in the L*c*h color scale, with difference in hue angle; 13° for fish and 20° for beef. No clear trend was found on the changes of the lightness of gummies over 24 days for both gel sources (Figure 1). However, the change of a* and b* value for BG and b* value only for FG was significant over 24 days (Figure 2 and 3). The decrease in a* value of BG indicated a less red-purple coloration over time. While the increment in b* value in both gummies represented the color of more yellowish, according to the CIE L*a*b* color scale. However, the anthocyanin concentration in gummies decreased at similar rates for FG and BG according to the almost similar percentage of final concentration after 24 days 57% or FG and 56% for BG (Figure 4). This suggested that the source of gelatin might not significantly affect chokeberry anthocyanins stability. It was noted that there was a significant decrease in the anthocyanin concentration in both at Day 17. It might be because of the incomplete anthocyanin extraction during the gelatin precipitation. The Delta E values over 24 days for FG was 6.48 and 5 for BG, which was hard to be noticed by an untrained observer.

For the AE extract, two hours after mixing, the color of FG was in the blue to red region in the L*c*h color scale, with hue angle of 340°, while the BG was in red to yellow color with the hue angle of 6°. The huge difference in the color might because of the difference in the initial color of the BG and FG gelatin solutions without adding the anthocyanin. For the lightness of FG and BG with AE extract, there were no significant differences for both FG and BG respectively over 36 days (Figure 5). The a* value ($P = 0$) only significant for the BG as how the chokeberry performed (Figure 6). The increase in a* value indicates of the similar trend in chokeberry, where there was less red purple coloration over time. Similarly with chokeberry, for the b*, both BG and FG significantly increased over 36 days (Figure 7). The increased in b* value represented the color of gummies more yellowish. The Delta E value over 36 days for FG was 4, hard to be noticed by an untrained observer, while it was 12 for the BG which means the color was more similar than the opposite. For the comparison of the delta E, the AE and chokeberry extracts showed different trend; both the FG and BG of chokeberry as well as FG of AE has value of less than 10. Only the BG of AE had a delta E value higher than 10.

The anthocyanin concentration for chokeberry decreased after 24 days with percentage of 57% for FG and 56% for BG (Figure 4). While it was 15% and 19% only for FG and BG

respectively for American elderberry after 36 days (Figure 8). It might be because of the high stability in American elderberry which has majority acylated anthocyanin. Acylated anthocyanin is more stable due to the naturally acylated pigment in the American elderberry which improved the pigment stability and proven to be more stable as compared to that of non-acylated cyanidin-based anthocyanins when tested in different temperatures over 90 days (Lee and Finn, 2007)

There are some limitations in this study where there need three replications of the anthocyanin quantification, beside a better approach on extracting anthocyanins from gelatin. However, this study would give the basis or idea on how the different acylation and gelatin sources could affect the color performance as well as the stability of the gummies itself.

Conclusion

Overall, the stability of the anthocyanins from American elderberry (80% acylated pigments) was better than the stability of chokeberry anthocyanin (all non-acylated), in agreement with our hypothesis of increased stability with acylated pigments.

The colors obtained with the different anthocyanin sources in beef gelatin were lighter and more yellow than in the fish gelatin. This also supports our hypothesis that the type of gel affects color expression.

While the color stability of chokeberry anthocyanins in the gelatins from the different sources was not significantly different, however, some differences were found in the color stability of gels colored with the American elderberry. Larger color changes were observed in beef gelatin colored with American elderberry (delta E > 10) than in fish gelatin containing the same pigment.

Based on our results, my recommendation for food industry is to use the fish gelatin since it has a darker and more stable color compared to the beef gelatin. The American elderberry also is suitable to use than the chokeberry due to the high anthocyanins stability.

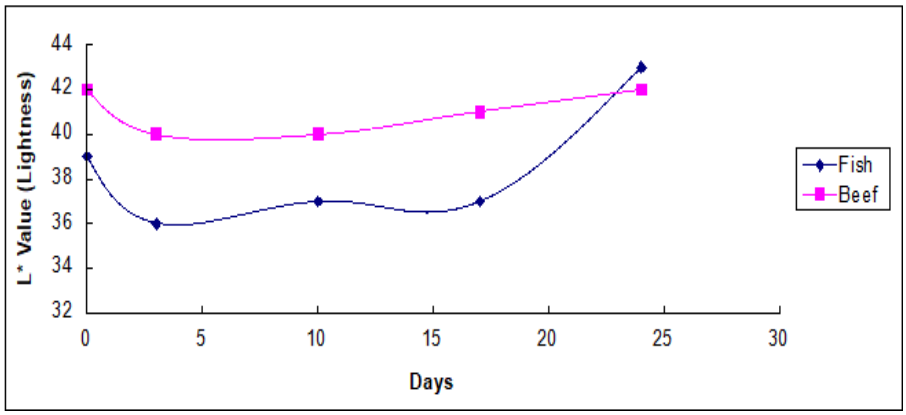

Figure 1 The trend of lightness of gummies colored with chokeberry over 24 days

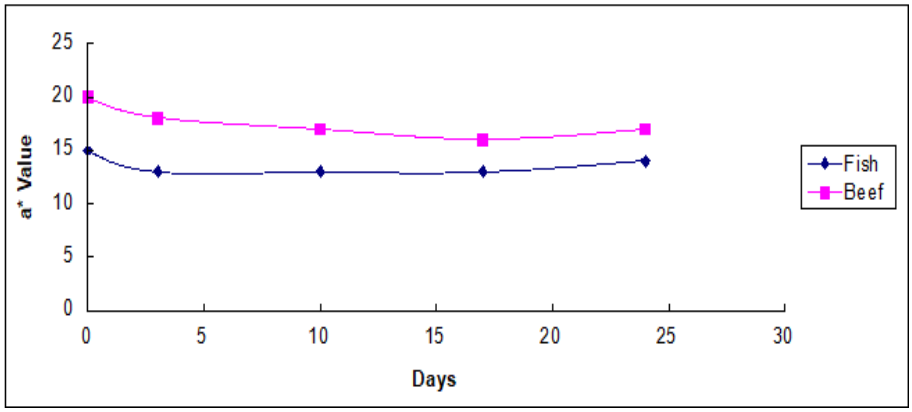

Figure 2 The trend of a* value of gummies colored with chokeberry over 24 days

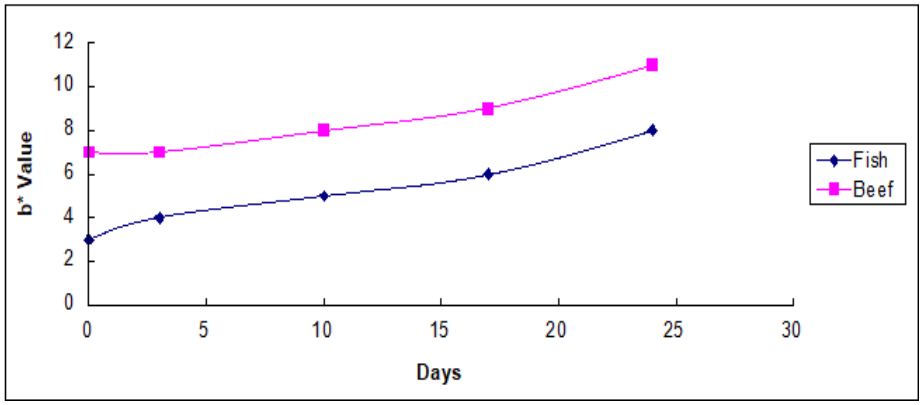

Figure 3 The trend of b* value of gummies colored with chokeberry over 24 days

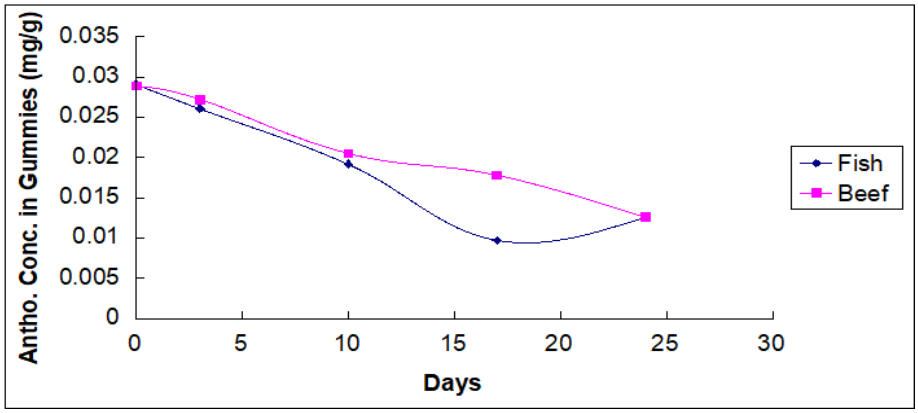

Figure 4 Changes in chokeberry anthocyanin concentration of gummies over 24 days

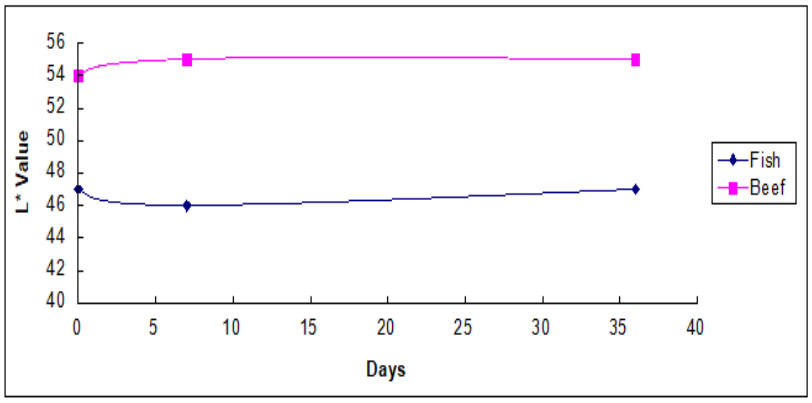

Figure 5 The trend of lightness of gummies colored with AE over 36 days

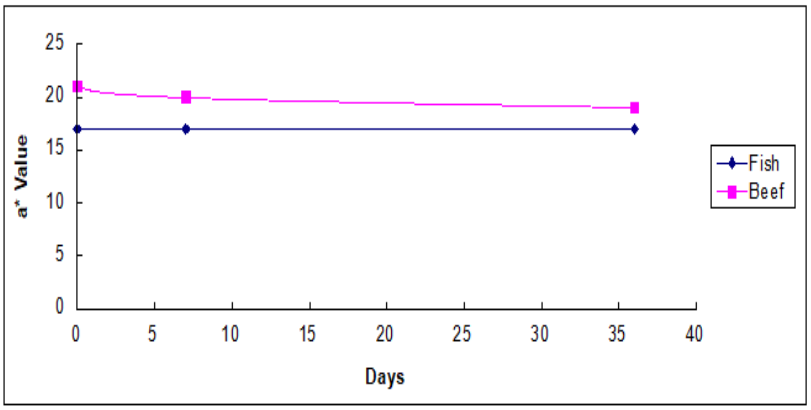

Figure 6 The trend of a* value of gummies colored with AE over 36 days

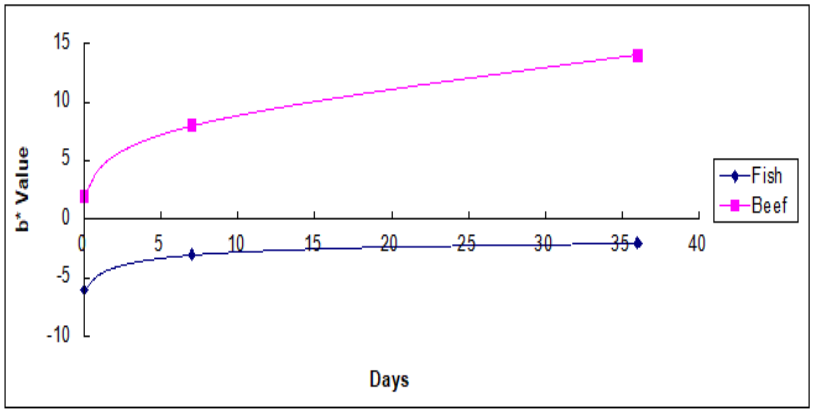

Figure 7 The trend of b* value of gummies colored with AE over 36 days

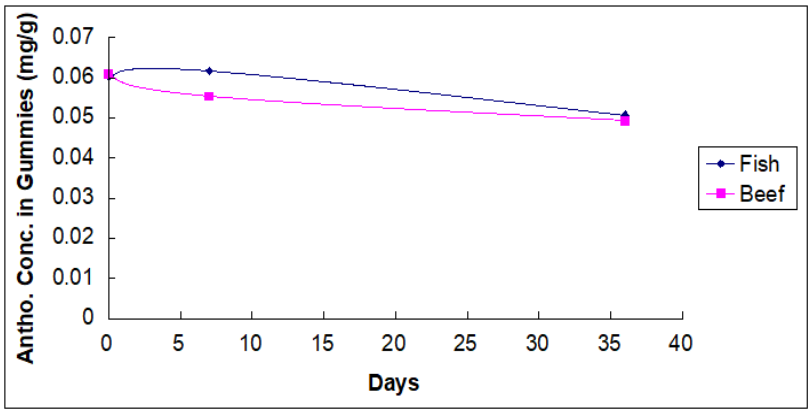

Figure 8 Changes in AE anthocyanin concentration of gummies over 36 days

References

Giusti, M. and Wrolstad, R. E. 2001. Characterization and Measurement of Anthocyanins by UV-Visible Spectroscopy. Current Protocols in Food Analytical Chemistry. F:F1:F1.2.

GrandViewResearch. 2017. Market Research Report. Natural Food Colors Market Estimates & Trend Analysis By Product (Curcumin, Carotenoids, Anthocyanin, Carmine, Chlorophyllin), By Application (Bakery & Confectionery, Beverages, Dairy & Frozen Products, Meat Products), And Segment Forecasts, 2014 – 2025. Available from: http://www.grandviewresearch.com/industry-analysis/natural-food-colors-market. Accessed 2017 November 15.

Imeson, A. P. (1997). *Thickening and Gelling Agents for Food*. Boston, MA: Springer US.

Jeya, S. R., Jeevithan, E., Varatharajakumar, A., Jeyasekaran, G., & Sukumar, D. (2012). Functional characterization of gelatin extracted from bones of red snapper and grouper in comparison with mammalian gelatin. *Lwt - Food Science and Technology*, *48*, 1, 30-36.

Lee, J., & Finn, C. E. (2007). Anthocyanins and other polyphenolics in American elderberry (*Sambucus canadensis*) and European elderberry (*S. nigra*) cultivars. Journal of the Science of Food and Agriculture, 87(14), 2665-2675. doi:10.1002/JSFA.3029

Li, D., Wang, P., Luo, Y., Zhao, M., & Chen, F. (2017). Health benefits of anthocyanins and molecular mechanisms: Update from recent decade. *Critical reviews in food science and nutrition*, *57*(8), 1729-1741.

Maier, T., Fromm, M., Schieber, A., Kammerer, D. R., & Carle, R. (2009). Process and storage stability of anthocyanins and non-anthocyanin phenolics in pectin and gelatin gels enriched with grape pomace extracts. *European Food Research and Technology : Zeitschrift Für Lebensmittel-Untersuchung Und Forschung A*, *229*, 6, 949-960.

Malien-Aubert, C., Dangles, O., & Amiot, M. J. (2001). Color stability of commercial anthocyanin-based extracts in relation to the phenolic composition. Protective effects by intra- and intermolecular copigmentation. Journal of Agricultural and Food Chemistry, 49, 1, 170-6.

Nur, H. Z. A., Roos, Y. H., & Kerry, J. P. (2012). Use of beef, pork and fish gelatin sources in the manufacture of films and assessment of their composition and mechanical properties. *Food Hydrocolloids*, *29*, 1, 144-151.

Vlachojannis, C., Zimmermann, B. F., & Chrubasik-Hausmann, S. (2015). Quantification of Anthocyanins in Elderberry and Chokeberry Dietary Supplements. Phytotherapy Research, 29, 4, 561-565.

Willig, J. (2009). *The Effect of Anthocyanin Acylation on the Inhibition of HT-29 Colon Cancer Cell Proliferation.* (Electronic Thesis or Dissertation). Retrieved from https://etd-ohiolink-edu.proxy.lib.ohio-state.edu/